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Single nucleotide polymorphisms of Caudal type homeobox 1 and 2 are associated with Barrett's esophagus

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Abstract

Background—Barrett's esophagus (BE), the premalignant lesion of esophageal adenocarcinoma, is believed to develop as a result of chronic gastroesophageal reflux disease (GERD). Approximately 10% of subjects with GERD progress to BE. Genetic, epigenetic and other risk factors may contribute to this inter-individual variability. *Caudal type homeobox 1* (*Cdx1)* and *Caudal type homeobox 2* (*Cdx2)* play important regulatory roles in the development of human BE.

Aims—To determine associations between *Cdx1* and *Cdx2* SNPs and BE

Methods—Genomic DNA was extracted from blood samples collected from BE (n = 109) and GERD (n = 223) patients for genotyping of 5 single nucleotide polymorphisms (SNPs) each of *Cdx1* and *Cdx2* using TaqMan allelic discrimination assays. Odds ratios (OR) and 95% confidence intervals (CI) of SNPs and haplotypes were calculated with a logistic regression model adjusted for factors including age, sex and hiatal hernia. Interactions between genetic variants and these three risk factors were also analyzed.

Results—Older age (50 years), male sex and hiatal hernia were significantly associated with BE ($P < 0.001$). Five variants of *Cdx1* SNPs (rs3776082, rs717746 and rs3776083), one *Cdx1* haplotype, and three variants of *Cdx2* SNPs (rs4769585 and rs3812863) were associated with BE ($P < 0.05$). Statistically significant interactions were detected between most of these SNPs and the three risk factors ($P < 0.05$).

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Conclusion—Certain SNPs of *Cdx1* and *Cdx2* and their interactions with other risk factors are associated with BE, and may contribute to human susceptibility to BE.

Keywords

Barrett's esophagus; gastroesophageal reflux disease; *Cdx1*; *Cdx2*; SNP

Introduction

Patients with chronic gastroesophageal reflux disease (GERD) are at a high risk for the development of Barrett's esophagus (BE), which is the premalignant lesion of esophageal adenocarcinoma defined by the presence of endoscopically evident, intestinalized columnar epithelium in the esophagus. Previous studies reported that approximately 10% of subjects with GERD progress to BE [1, 2]. Although it is not clear why some individuals are susceptible to BE, there is increasing evidence to suggest that genetic factors play critical roles in the development of BE. BE tends to cluster in families and identical twins [3]. Using genomic linkage analysis, a number of studies have found several genomic regions or positional candidate genes associated with BE, such as *MSR1*, *ASCC1* and *CTHRC1* [4]. Variants of functional candidate genes have also been significantly associated with BE, including *IL-10* [5], *GSTP1* [6] and *IGF1* [7].

As members of the Caudal-related homeobox gene family, *Cdx1* and *Cdx2* are important for intestinal development. Since BE is characterized by intestinal differentiation, it is believed that *Cdx1* and/or *Cdx2* are essential for the development of BE [8]. *Cdx2* is not expressed in squamous epithelial cells of normal human esophagus, whereas it is expressed in both goblet and non-goblet cells of human BE [9]. *In vitro* studies show that *Cdx2* is activated in esophageal keratinocytes by acid or bile acids [10]. *Cdx1* is preceded by *Cdx2* during metaplastic conversion [11]. Transgenic overexpression of *Cdx1* or *Cdx2* induces intestinal metaplasia in the transgenic mouse stomach, though with slightly different phenotypes [12]. It is believed that *Cdx1* and *Cdx2* have mostly overlapping, but also certain distinct, functions.

A recent study reported that acid and bile salts induced *Cdx2* expression in the esophageal squamous cells obtained from BE, but not in those obtained from GERD without BE [13]. These data suggested that genetic variations of *Cdx2* may contribute to gene expression and thus susceptibility to BE. To date, no study has investigated the association of *Cdx1* and *Cdx2* variants with BE. Single nucleotide polymorphism (SNP) is a DNA sequence variation occurring when a single nucleotide in the genome differs between individuals in a population. In addition, a set of SNPs may associate with each other to be inherited together as a haplotype. Both SNPs and haplotypes may impact gene expression and function, and therefore underlie differences in our susceptibility to disease [14]. This pilot study was aimed to determine associations of the SNPs and haplotypes of these two intestinal genes with BE, and their interactions with sex, age and hiatal hernia.

Materials and Methods

Study subjects and recruitment

BE and GERD subjects were recruited at the University of North Carolina (UNC) Hospitals between June 2002 and January 2008. For inclusion, participants had to have BE with either no dysplasia or a single past transient reading of low-grade dysplasia (LGD). BE was defined as any endoscopically detectable upward displacement of the squamocolumnar junction in the tubular esophagus of greater than 5mm, with at least one biopsy specimen from the area demonstrating specialized columnar epithelium with goblet cells on routine

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pathology specimens. Subjects with goblet cells on biopsy, but no endoscopic appearance of BE, were not eligible for inclusion. Transient LGD was defined as a reading of LGD on a single biopsy specimen, with at least one subsequent endoscopy negative for dysplasia. Participants were excluded if they had a history of high-grade dysplasia (HGD) or adenocarcinoma, endoscopic ablation of BE, esophageal resection, or an active malignancy (except non-melanoma skin cancer). Erosive esophagitis (EE) was defined as endoscopically visible mucosal breaks with appreciable depth. Endoscopy-negative reflux disease (ERD) was diagnosed if patients reported substernal chest burning and/or regurgitation, but demonstrated no visible esophageal mucosal injury on endoscopy.

Blood samples were obtained for DNA extraction using a FlexiGene DNA Kit (Qiagen, Valencia, CA). Biopsy specimens were snap-frozen in liquid nitrogen for histopathological testing. Demographic and clinical information was recorded, including ethnicity, race, age, sex, hiatal hernia status and endoscopy history. The UNC Institutional Review Board approved this study.

Selection of SNPs and genotyping

A 10-kb 5′-flanking region of *Cdx2* and a 16-kb 5′-flanking region of *Cdx1* were scanned for known SNPs [15, 16]. In addition, to evaluate potential regulatory elements in the 3′UTR, 4 kb downstream of the stop codons of both *Cdx1* and *Cdx2* were included. All SNPs of *Cdx1* and *Cdx2* genes were selected from the database of the International HapMap Project (data release #28). Shannon's entropy was calculated to evaluate genetic diversity based on the genotyping data of CEU from HapMap. Entropy-based SNP selection was utilized for genetic association studies. Final SNP selections were based on relatively high Shannon's entropy and genomic positions. In addition, two SNPs (rs9257809 and rs9936833) were included in this study based on their associations with BE in a recent genome-wide association study [17].

Genotyping was performed using 5′ nuclease TaqMan assays pre-designed by Applied Biosystems (Foster City, CA). All TaqMan assay primers of 12 SNPs are available on the website of the manufacturer (http://www.lifetechnologies.com/us/en/home.html). PCR reactions in 384-well plates were carried out on the ABI 9700 thermal cycler (Applied Biosystems) using 5 ng purified DNA as the template. Genotype was detected using an ABI PRISM 7900 HT Sequence Detection System with SDS 2.3 software (Applied Biosystems).

Statistical analysis

Age, hiatal hernia and sex-corrected odds ratios (ORs), 95% confidence intervals (95% CIs) and gene-risk factor interactions of SNPs were analyzed by a logistic regression model in which the homozygous wild-type genotype was used as the reference. The Hardy-Weinberg equilibrium test, linkage disequilibrium analysis, haplotype analysis and gene-risk factor interaction analysis were performed online (http://bioinfo.iconcologia.net/snpstats/start.htm) [17]. A standard Bonferroni correction was used to adjust for multiple testing. The most frequent haplotype was automatically selected as the reference category, and a global test for interaction was performed online.

Results

Three hundred and thirty-two Caucasian subjects were recruited, including 109 BE, 31 EE and 192 ERD subjects. The majority of BE subjects were older than 50 (79.8%), male (66.1%) and complicated with hiatal hernia (64.2%) (Table 1). EE and ERD were pooled together as GERD for comparison with BE.

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A 37.63-kb region (chr5:149,510,537 – 149,548,165) and a 21.04-kb region (chr13:27,430,279 – 27,451,317) were scanned for SNPs in HapMap. Fifty-two *Cdx1* SNPs and 21 *Cdx2* SNPs were found. Based on the criteria reviewed above, 10 SNPs were selected, including 5 SNPs each of *Cdx1* and *Cdx2* (Table 2). The distribution of genotype frequencies for the selected SNPs was consistent with Hardy-Weinberg equilibrium in all subjects ($P > 0.05$). All SNPs were in linkage disequilibrium except for rs3776083 and rs3756311 in *Cdx1*.

Five variants of *Cdx1* SNPs (rs3776082, rs717746 and rs3776083) and three variants of *Cdx2* SNPs (rs4769585 and rs3812863) were associated with BE ($P < 0.05$, Table 2). Three variants of *Cdx1* were associated with BE after Bonferroni correction. Several SNP variants, including the significant SNPs, significantly interacted with the other BE risk factors and showed an increased association with BE among subjects possessing both the risk factor and the SNP variant after Bonferroni correction (Table 3).

Ten *Cdx1* haplotypes and eight *Cdx2* haplotypes were observed. One of the *Cdx1* haplotypes (GAGGA) was significantly associated with BE (OR 2.27, 95% CI 1.14–4.54, $P = 0.02$) (Table 4). Additionally, haplotypes of *Cdx1* interacted with male sex significantly ($P = 0.0068$) (Table 5).

For SNPs rs9257809 and rs9936833, no significant association with BE was observed (Table 2). However, the wild-type *A* allele of rs9257809 and the mutant *C* allele of rs9936833 showed significant interactions with risk factors including male, older age and hernia (Table 3).

Discussion

This study is a pilot study to assess the association between SNPs of *Cdx1* and *Cdx2* and BE. Based on clinical data, BE population was older and having more hiatal hernia and male subjects than GERD controls (Table 1). Five SNPs and 1 haplotype of *Cdx1* and *Cdx2* were associated with BE adjusted for sex, age and hiatal hernia (Table 2 and Table 4). Significantly positive interactions between SNP variants and these risk factors were also observed (Table 3).

Two SNPs (rs4769585 and rs3812863) and one haplotype of *Cdx2* were significantly associated with BE. Although these SNPs are located within 7.2-kb and 1.9-kb 5′-flanking regions, respectively, it is not clear whether they may impact gene expression or functions. Expression of *Cdx2* is known to be regulated by promoter methylation [10] and NF-κB [13, 19]. Treatment of human esophageal epithelial cells with acid or bile partially demethylates the *Cdx2* CpG island (−1,769 bp to −1,363 bp) and markedly activates its expression [10]. However, this regulatory mechanism by promoter methylation was not reproduced in another independent study. Use of different cell lines may contribute to the discrepancy [10, 13]. For NF-κB, there is a stepwise increased activity in human GERD and BE [20]. Activation of NF-κB induces *Cdx2* expression in human gastroesophageal junction adenocarcinoma cells [21]. Recently, two independent studies demonstrated that NF-κB binding sites (−103 bp to −82 bp and −22 bp to −1 bp) in the *Cdx2* promoter were responsible for the bile acid-induced activation [13, 19].

Genetic variants may explain differential expression of *Cdx2* human subjects with gastroesophageal reflux. *Cdx2* expression was found in esophageal squamous epithelium in 6 of 19 biopsy specimens from subjects with GERD and BE, but in none of 40 GERD subjects without BE [22]. Acid and bile salts induce *Cdx2* expression in esophageal squamous cells from subjects with BE, but not in those cells from GERD subjects without

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BE [13]. One SNP (rs3812863) in *Cdx2* promoter occurred in the binding site of forkhead box protein P1, which is a transcriptional repressor and plays an important role in lung and esophageal development [23]. Further studies are needed to identify genetic variants in the promoter region which may in part dictate *Cdx2* expression in esophageal epithelial cells.

Three SNPs (rs3776082, rs717746 and rs3776083) and one haplotype (GAGGA) of *Cdx1* were significantly associated with BE. Rs3776082 is located in the 2.3-kb 5′-flanking region, rs717746 in intron 1, and rs3776083 in the 3′-flanking region. Similar to *Cdx2*, the 5′UTR region of *Cdx1* has at least four potential NF-κB subunit binding sites through which NF-κB subunits may activate *Cdx1* expression directly [24]. In fact, transgenic expression of the nucleotides −15,601 to +68 of *Cdx1* gene was restricted to the intestinal epithelium, suggesting that this region contains intestine-specific enhancers, which are short regions of DNA that can be bound by transcription factors to enhance transcription levels of intestinal genes [16].

Consistent with previous results, older age (≥50 years), male sex and hiatal hernia were associated with BE in this study [25, 26]. Many SNPs in this study significantly positively interacted with these risk factors ($P < 0.05$, Table 3). In addition to SNP variants, *Cdx1* haplotypes also significantly interacted with sex ($P < 0.01$).

In addition to Caucasian subjects, 41 African-Americans were enrolled in this study, including 1 BE, 5 EE and 35 ERD. In our preliminary analysis including race as an additional covariate, Caucasian race was very significantly associated with BE ($P < 0.05$, data not shown). Association analysis with SNPs was not performed due to the small sample size and imbalanced data set.

Recently, the first genome-wide association study on BE was reported [17]. Variants at two loci were associated with BE: chromosome 6p21, rs9257809 ($P_{combined} = 4.09 \times 10^{-9;}$ OR = 1.21, 95%CI =1.13–1.28) and chromosome 16q24, rs9936833 ($P_{combined} = 2.74 \times 10^{-10}$; OR = 1.14, 95% CI = 1.10–1.19). However, no significant association with BE was observed in this study (Table 2), with the exception of marked interaction between the wild-type *A* allele of rs9257809 and the mutant *C* allele of rs9936833 and risk factors (Table 3), indicating the complex genetic susceptibility to BE. We speculate that different ethnic or geographic populations may account for such discrepancy.

Several limitations of our work deserve mention. Our study population was predominantly Caucasian. While previous data on BE prevalence similarly indicate an overwhelming preponderance in Caucasians, the generalizability of our data is limited. Secondly, subjects were not required to stop taking acid suppressive medications to participate in the trial. This likely caused some patients who would have otherwise been categorized as EE to become ERD. The logistical problems with manipulating subjects' medications in advance of their enrollment in the study, as well as the ethical issues involved in stopping acid-suppressive medications in subjects with BE, made stopping acid suppression untenable. Thirdly, BE group was significantly older and having more male subjects and hiatal hernia than GERD group. Therefore our study may potentially identify correlations between these risk factors and BE, instead of causative risk. Fourthly, there is a small chance that the controls may develop BE over time. This may impact our findings to a certain extent. Lastly, this study has a relatively small sample size and is prone to type II error. Nevertheless, these novel findings are preliminary and must be confirmed in subsequent studies with larger cohorts. We are currently recruiting a confirmation cohort for an expression quantitative trait loci (eQTL) study to associate these SNPs and haplotypes with gene expression [27]. North Carolina has a relatively high African-American population, and recruitment of more

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African-American patients will allow us to further understand the potential contribution of genetic variants in racial disparity of BE [28].

In conclusion, 5 SNPs and 1 haplotype of *Cdx1* and *Cdx2* were significantly associated with BE. Significant interactions were observed between SNPs and other risk factors.

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Table 1

Characteristics of clinical subjects in this study

Characteristic	BE (n = 109)	ERD (n = 192)	EEE (n = 31)	GERD (ERD + EE) (n = 223)
Sex, male *	72 (66.1%)	77 (40.1%)	15 (48.4%)	92 (41.3%)
Hiatal hernia *	70 (64.2%)	76 (39.6%)	21 (67.7%)	97 (43.5%)
Age (years) *				
≥50	87 (79.8%)	103 (53.6%)	21 (67.7%)	124 (55.6%)
Range	28 – 81	19 – 80	20 – 74	19 – 80
Mean ± SD	59.2 ± 10.8	51.4 ± 13.9	52.6 ± 12.8	51.5 ± 13.7

* Significant difference between GERD and BE according to Fisher's exact test ($P < 0.001$).

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Table 2

Association between *Cdx1* and *Cdx2* SNP variants and BE using homozygous wild-type genotypes as references

Gene	SNP	Allele (wild- type[#])	Position	Shannon's entropy	Genotype (No. of subjects, BE/GERD)	Frequency	OR (95% CI)	*P* value
Cdx1	rs3756311	A/G (GG)	5′ region	0.98	AG (47/100)	0.45	0.77 (0.37 – 1.57)	0.46
					AA (41/82)	0.38	0.71 (0.34 – 1.49)	0.37
	rs3776082	A/G (GG)	Promoter	1.03	AG (61/117)	0.54	2.54 (1.23 – 5.24)	0.01*
					AA (35/45)	0.24	4.02 (1.80 – 9.00)	< 0.01**
	rs2237091	A/G (GG)	Intron 1	1.02	AG (53/258)	0.50	0.58 (0.32 – 1.04)	0.07
					AA (15/61)	0.24	0.28 (0.13 – 0.59)	< 0.01**
	rs717746	G/T (TT)	Intron 1	1.02	GT (54/108)	0.49	2.07 (1.05 – 4.08)	0.04*
					GG (39/47)	0.26	3.65 (1.73 – 7.69)	< 0.01**
	rs3776083	A/G (GG)	3′ region	1.02	AG (62/93)	0.49	1.91 (1.09 – 3.33)	0.02*
					AA (11/23)	0.11	1.47 (0.61 – 3.54)	0.39
Cdx2	rs4769585	C/T (TT)	5′ region	0.98	CT (59/106)	0.53	2.68 (1.20 – 5.98)	0.02*
					CC (37/62)	0.32	2.56 (1.10 – 5.94)	0.03*
	rs3812863	A/G (GG)	Promoter	1.01	AG (62/107)	0.52	2.53 (1.24 – 5.14)	0.01*
					AA (30/63)	0.28	1.95 (0.89 – 4.24)	0.09
	rs2504211	T/G (GG)	Intron 2	0.73	GT (41/76)	0.38	1.17 (0.69 – 2.00)	0.56
					TT (3/14)	0.05	0.37 (0.10 – 1.40)	0.14
	rs1805107	A/G (GG)	Exon 3	0.73	AG (41/76)	0.36	3.11 (0.80 – 12.12)	0.10
					AA (63/125)	0.58	2.45 (0.65 – 9.32)	0.19
	rs2481952	T/C (CC)	3′ UTR	1.00	CT (57/110)	0.51	1.53 (0.82 – 2.84)	0.18
					TT (28/48)	0.23	1.77 (0.86 – 3.64)	0.12
Unknown	rs9257809	A/G (AA)	6p21	1.01	AG(14/26)	0.13	1.05(0.49 – 2.23)	0.90
					GG(0/5)	0.02	0 (0 – ∞)	0.99
Unknown	rs9936833	T/C (TT)	16q24	1.00	TC(45/86)	0.41	1.21(0.70 – 2.10)	0.50
					CC(22/31)	0.16	1.52(0.75 – 3.10)	0.25

*Significant *P* value without correction for multiple testing.

**Significant *P* value after Bonferroni correction (α = 0.05/10 or 0.005).

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#Wild-type alleles are shown in parentheses.

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Table 3

Interactions between *Cdx1* and *Cdx2* genetic variants and the risk factors of BE

Gene	SNP	Genotype[#]	Male OR (95% CI)	*P* value	≥50 years OR (95% CI)	*P* value	Hernia OR (95% CI)	*P* value
Cdx1	rs3756311	GG	0.63 (0.18 – 2.21)	0.47	2.12 (0.59 – 7.61)	0.25	1.26 (0.37 – 4.27)	0.71
		AG	1.70 (0.70 – 4.09)	0.24	1.74 (0.56 – 5.40)	0.34	1.3 (0.46 – 3.69)	0.62
		AA	1.12 (0.46 – 2.73)	0.81	1.97 (0.63 – 6.15)	0.24	0.99 (0.33 – 2.96)	0.98
	rs3776082	GG	6.70 (1.33 – 33.84)	0.02*	7.75 (0.92 – 64.99)	0.06	0.59 (0.16 – 2.15)	0.42
		AG	13.60 (3.01 – 61.43)	<0.001**	19.73 (2.54 – 153.09)	0.004**	3.44 (1.37 – 8.61)	0.01*
		AA	19.01 (3.93 – 92.01)	<0.001**	19.57 (2.41 – 158.85)	0.005**	3.96 (1.40 – 11.14)	0.01*
	rs2237091	GG	2.76 (1.08 – 7.05)	0.03*	1.63 (0.61 – 4.31)	0.33	1.6 (0.63 – 4.06)	0.32
		AG	1.62 (0.69 – 3.78)	0.27	1.22 (0.49 – 3.01)	0.67	1.16 (0.51 – 2.65)	0.72
		AA	0.85 (0.31 – 2.37)	0.76	0.66 (0.24 – 1.87)	0.44	0.37 (0.13 – 1.09)	0.07
	rs717746	TT	2.80 (0.84 – 9.33)	0.09	11.77 (1.43 – 96.55)	0.02*	0.85 (0.27 – 2.69)	0.78
		GT	5.92 (2.05 – 17.1)	0.001**	21.09 (2.71 – 163.83)	0.003**	3.19 (1.27 – 8.03)	0.01*
		GG	10.15 (3.25 – 31.7)	<0.001**	29.10 (3.63 – 233.22)	0.001**	3.93 (1.42 – 10.89)	0.01*
	rs3776083	GG	2.68 (1.11 – 6.44)	0.03*	4.96 (1.82 – 13.53)	0.001**	2.73 (1.14 – 6.54)	0.02*
		AG	5.09 (2.16 – 12.00)	<0.001**	8.43 (3.20 – 22.22)	<0.001**	4.24 (1.84 – 9.74)	<0.001**
		AA	7.33 (2.17 – 24.70)	0.001**	3.69 (0.91 – 14.99)	0.07	3.28 (0.99 – 10.93)	0.05*
Cdx2	rs4769585	TT	1.94 (0.47 – 8.04)	0.36	1.91 (0.34 – 10.71)	0.46	9.86 (1.10 – 88.31)	0.04*
		CT	5.70 (1.71 – 18.95)	0.004**	6.68 (1.36 – 32.78)	0.02*	15.24 (1.88 – 123.34)	0.01*
		CC	7.06 (2.03 – 24.64)	0.002**	4.58 (0.91 – 23.00)	0.06	21.54 (2.57 – 180.76)	0.004**
	rs3812863	GG	1.35 (0.40 – 4.55)	0.62	∞ (0 – ∞)	0.99	10.22 (2.00 – 52.29)	0.005**
		AG	4.92 (1.74 – 13.88)	0.002**	∞ (0 – ∞)	0.98	12.53 (2.74 – 57.36)	0.001**
		AA	3.99 (1.31 – 12.10)	0.01*	∞ (0 – ∞))	0.98	12.94 (2.69 – 62.30)	0.001**
	rs2504211	GG	2.37 (1.22 – 4.62)	0.01*	3.98 (1.83 – 8.68)	<0.001**	1.61 (0.83 – 3.15)	0.16
		GT	3.67 (1.70 – 7.92)	<0.001**	4.25 (1.83 – 9.86)	<0.001**	2.31 (1.06 – 5.01)	0.03*
		TT	1.07 (0.19 – 6.09)	0.94	1.13 (0.20 – 6.28)	0.89	1.03 (0.24 – 4.56)	0.96
	rs1805107	GG	3.94 (0.27 – 58.07)	0.32	1.57 (0.10 – 23.87)	0.75	∞ (0 – ∞)	0.98

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Gene	SNP	Genotype[#]	Male OR (95% CI)	*P* value	≥50 years OR (95% CI)	*P* value	Hernia OR (95% CI)	*P* value
		AG	12.75 (1.42 – 114.42)	0.02*	5.67 (0.58 – 55.42)	0.14	∞ (0 – ∞)	0.98
		AA	8.00 (0.92 – 69.22)	0.06	5.02 (0.53 – 47.82)	0.16	∞ (0 – ∞)	0.98
	rs2481952	CC	2.89 (1.00 – 8.38)	0.05*	4.89 (1.43 – 16.79)	0.01*	8.30 (2.16 – 31.92)	0.002**
		CT	4.51 (1.72 – 11.83)	0.002**	6.53 (2.06 – 20.72)	0.001**	7.32 (2.02 – 26.60)	0.002**
		TT	5.00 (1.72 – 14.56)	0.003**	7.42 (2.12 – 26.06)	0.001**	8.89 (2.26 – 35.07)	0.002**
Unknown	rs9257809	AA	2.84 (1.62 – 4.96)	0.001**	2.94 (1.57 – 5.52)	0.001**	2.29 (1.31 – 4.01)	0.004**
		AG	3.61 (1.35 – 9.64)	0.01*	3.39 (1.25 – 9.17)	0.02*	2.07 (0.77 – 5.58)	0.15
		GG	∞ (0 – ∞)	0.99	∞ (0 – ∞)	0.99	∞ (0 – ∞)	0.99
Unknown	rs9936833	TT	2.38 (1.09 – 5.22)	0.03*	1.90 (0.82 – 4.39)	0.13	2.27 (1.03 – 4.98)	0.04*
		TC	3.95 (1.80 – 8.70)	0.001**	2.69 (1.16 – 6.21)	0.02*	2.48 (1.15 – 5.34)	0.02*
		CC	2.99 (1.13 – 7.98)	0.03*	4.70 (1.72 – 12.88)	0.003**	3.35 (1.32 – 8.52)	0.01*

*Significant *P* value without correction for multiple testing;

**Significant *P* value after Bonferroni correction (α = 0.05/10 or 0.005);

[#]The first genotype of each SNP is a wild-type allele.

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Table 4

Association between major haplotypes of *Cdx1* and *Cdx2* and BE (adjusted for age, sex and hernia)

Gene	Haplotype	Frequency	OR (95% CI)	*P* value
Cdx1	AGATG	0.29	1.00	---
	AAGGA	0.16	1.86 (0.95 – 3.62)	0.07
	GAGGA	0.13	2.27 (1.14 – 4.54)	0.02*
	GGATG	0.11	0.83 (0.36 – 1.91)	0.66
	GAGGG	0.10	1.41 (0.64 – 3.09)	0.39
	AAGGG	0.08	2.13 (0.86 – 5.26)	0.10
	AGATA	0.04	0.69 (0.16 – 3.00)	0.62
	GAATG	0.03	2.34 (0.75 – 7.24)	0.14
	GGATA	0.02	0.52 (0.05 – 5.25)	0.58
	AGGGG	0.02	1.07 (0.25 – 4.61)	0.93
Cdx2	CAGAT	0.33	1.00	---
	TGGAC	0.25	0.58 (0.35 – 0.95)	0.03*
	CGTGC	0.13	0.71 (0.38 – 1.35)	0.30
	TAGAT	0.12	0.52 (0.25 – 1.09)	0.08
	CATGC	0.09	0.61 (0.30 – 1.25)	0.18
	TGGAT	0.03	0.62 (0.22 – 1.74)	0.37
	CGGAC	0.03	0.44 (0.11 – 1.69)	0.23
	TGTGC	0.02	0.79 (0.13 – 4.64)	0.79

*Significant *P* value without correction for multiple testing.

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Table 5

Interactions between *Cdx1* and *Cdx2* haplotypes and the risk factors of BE

Gene	Haplotype	Male OR (95% CI)	*P* value	≥50 years OR (95% CI)	*P* value	Hernia OR (95% CI)	*P* value
Cdx1	AGATG	6.05 (0.76 – 48.25)	0.0068*	11.90 (1.52 – 93.45)	0.51	2.84 (0.64 – 12.58)	0.26
	AAGGA	21.53 (2.88 – 161.02)		18.25 (2.43 – 136.95)		6.19 (1.56 – 24.54)	
	GAGGA	8.78 (1.13 – 68.29)		16.22 (2.05 – 128.33)		5.00 (1.12 – 22.31)	
	GGATG	6.87 (0.92 – 51.24)		8.77 (1.10 – 69.97)		2.36 (0.55 – 10.14)	
	GAGGG	5.06 (0.58 – 44.44)		14.11 (1.69 – 117.71)		6.83 (1.32 – 35.36)	
	AAGGG	8.99 (1.16 – 69.61)		22.25 (2.71 – 182.75)		3.40 (0.72 – 16.11)	
	AGATA	3.40 (0.28 – 40.92)		9.27 (0.86 – 100.43)		∞ (0 – ∞)	
	GAATG	10.58 (0.77 – 144.66)		26.84 (2.76 – 261.44)		5.70 (0.95 – 34.16)	
	GGATA	∞ (0 – ∞)		11.13 (0.48 – 256.76)		12.34 (0.17 – 919.29)	
	AGGGG	7.22 (0.62 – 84.21)		11.06 (0.75 – 163.61)		∞ (0 – ∞)	
Cdx2	CAGAT	5.17 (1.51 – 17.73)	0.26	3.49 (0.94 – 12.90)	0.25	0.98 (0.30 – 3.24)	0.61
	TGGAC	2.26 (0.78 – 6.53)		2.40 (0.72 – 8.01)		0.71 (0.25 – 2.07)	
	CGTGC	3.00 (0.90 – 10.01)		2.55 (0.71 – 9.10)		0.91 (0.28 – 2.97)	
	TAGAT	1.46 (0.40 – 5.40)		1.62 (0.43 – 6.16)		0.58 (0.18 – 1.91)	
	CATGC	4.43 (1.25 – 15.65)		1.26 (0.30 – 5.26)		0.80 (0.23 – 2.73)	
	TGGAT	2.39 (0.49 – 11.72)		1.04 (0.14 – 7.54)		0.39 (0.05 – 2.81)	
	CGGAC	0.82 (0.12 – 5.75)		1.76 (0.31 – 10.00)		2.32 (0.19 – 28.35)	
	TGTGC	3.26 (0.23 – 46.48)		3.53 (0.38 – 32.44)		0.84 (0.07 – 10.05)	

*Significant *P* value without correction for multiple testing.